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10 November 1994 (10.11.94)

(54) Title: FLUOROCARBON COMPOSITIONS CONTAINING A VISIBLE OR FLUORESCENT LABEL

(57) Abstract

A composition for use in visualizing tissues comprising a physiologically-acceptable fluorocarbon liquid and a visualizable label such as a chromophore or visible or fluorescent dye associated therewith; preferably the fluorocarbon is in the form of an emulsion and the label has a lipophilic moiety. Also disclosed are methods for labeling and visualizing cells and tissue, such as those of the reticuloendothelial system.

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AMENDED CLAIMS

[received by the International Bureau on 6 September 1994 (06.09.94); original claim 1 amended; remaining claims unchanged (1 page)]

1. A fluorocarbon composition for use in visualizing cells or tissue of an animal, comprising:

a physiologically-acceptable liquid fluorocarbon; and

a visible or fluorescent label associated with said fluorocarbon.

2. The composition of Claim 1, further comprising:

an aqueous phase; and

an emulsifier, wherein said composition is an emulsion and said liquid fluorocarbon comprises a fluorocarbon phase of said emulsion.

3. The composition of Claim 2, wherein said label is a visible dye.

4. The composition of Claim 2, wherein said label is hydrophobic and is associated with said fluorocarbon phase or with said emulsifier by lipophilic or hydrophobic interacdtion.

5. The composition of Claim 2, wherein said label is a visible dye.

6. A method for visualizing cells or tissue of an animal, comprising the steps of:

administering to said animal *in vivo*, a highly fluorinated liquid fluorocarbon having a visible or fluorescent label associated therewith, and permitting said composition to localize in cells or tissue; and

illuminating said cells or tissue with visible or ultraviolet light so as to visualize said cells or tissue in which said fluorocarbon has localized.

7. The method of Claim 6, wherein said fluorocarbon composition is an emulsion and further comprises a continuous aqueous phase and an emulsifier, and wherein said liquid fluorocarbon comprises a discontinuous phase of said emulsion.

8. The method of Claim 7, wherein said discontinuous phase localizes in tissue of the reticuloendothelial system.

9. The method of Claim 8, wherein said label is a fluorescent label.

STATEMENT UNDER ARTICLE 19

Chowdhary et al. (*Photochem. Photobiol.*, 1990) is not relevant to the claims as amended since there is no disclosure or suggestion of the use of a dye-associated fluorocarbon for imaging cells or tissues in an animal.

European Patent application EP 0 272 933 A2 describes a process for the preparation of an information recording medium which involves, in part, mixing a cyanine dye with a perfluorinated alcohol. There is no disclosure pertaining to use of this mixture in imaging cells or tissues. Further, this reference is nonanalogous art since it is not directed to biological systems. Thus, one of ordinary skill in the art would not have consulted such a reference to determine a method for imaging cells or tissues.

Fed Proc. 34(6), 1493-1498, (1975) discloses an experiment in which NADH fluorescence is measured in mice transfused with a fluorocarbon emulsion to determine the effect of oxygen deprivation and restoration of brain NADH level. There is no teaching or

suggestion that the NADH is associated with the fluorocarbon; the NADH is simply used as a marker to measure the effect of the FC emulsion transfusion.

Prog. Colloid Polymer Sci., 76 (1988) teaches fluorescent dye incorporation into fluorocarbon surfactant-rich micelles. There is no motivation provided to use these micelles in imaging cells or tissues.

J. Am. Coll. Cardiol., 18(1), (1991) describes a study assessing the effects of blood-free reperfusion with intracoronary perfluorocarbon using IV injection of a fluorescent dye which stains the endothelium of blood vessels. The dye is not associated with the fluorocarbon.

Arch. Opthamol., 110(10), 1468-1471 (1992) only discloses the fluorescence spectra of perfluorocarbons. There is neither disclosure of perfluorocarbon association with a dye, nor imaging cells or tissues.

The *Gastroenterol. Endosc.*, *Cell Biol. Intl. Rep.*, and *J. Cell Biol.* references disclose the uptake of various dyes by cells and tissues. No fluorocarbons are taught or suggested by any of these references. The *J. Toxicol. Environ. Health* reference discloses perfluorinated fatty acids, but only in reference to their inhibition of MC540 dye binding by cells.

Thus, the claims as amended possess novelty and inventive step over the cited "X" references since none of these references teach or suggest imaging cells or tissues with a liquid fluorocarbon associated with a visible or fluorescent dye. Furthermore, there is no suggestion or motivation provided to combine any of the cited "Y" references to arrive at the claimed invention since none of these references disclose liquid fluorocarbons. In view of this, a labelled fluorocarbon for use in imaging cells or tissues and the imaging of cells or tissues with a dye-associated fluorocarbon certainly possess inventive step over any combination of these references. The amended claims thus patentably define over the prior art

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(57) Abstract

A composition for use in visualizing tissues comprising a physiologically-acceptable fluorocarbon liquid and a visualizable label such as a chromophore or visible or fluorescent dye associated therewith; preferably the fluorocarbon is in the form of an emulsion and the label has a lipophilic moiety. Also disclosed are methods for labeling and visualizing cells and tissue, such as those of the reticuloendothelial system.

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FLUOROCARBON COMPOSITIONS, CONTAINING A VISIBLE OR FLUORESCENT LABEL

Field of the Invention

The present invention relates to fluorocarbon compositions containing a visualizable label, and to their use in visualizing animal cells or tissues.

Background of the Invention

Liquid fluorocarbons have a high affinity for oxygen and can dissolve or solubilize significant quantities of oxygen. For this reason, fluorocarbon emulsions have been used as biocompatible oxygen carriers and/or blood substitutes.

Fluorocarbon liquids have also been used as radiological imaging agents. See e.g., U.S Patent Nos. 3,975,512 and 4,987,154. Fluorocarbon liquids have also been used in magnetic resonance imaging. See e.g., U.S. Patent No. 4,951,673.

In many instances it is desirable to directly visualize animal tissues or cells. Staining of tissues with tissue-specific dyes is a common histological technique. However, for certain tissues and cells, such as those reticuloendothelial (RES) system, there remains a need for better visualization techniques. A surgeon, for example, may use a radiological lymphographic technique to prepare pre-surgical films or pictures which indicate the location of lymphatic tissue in a patient. However, during surgery, working in the surgical incision, it may be more difficult to differentiate the tissue in question.

Similarly, in research applications, it is often desirable to identify and label certain tissues, such as RES tissues.

The present invention provides a ready mechanism for locating and visually identifying certain cells and tissues.

SUMMARY OF THE INVENTION

One aspect of the present invention is a fluorocarbon composition, comprising a physiologically-acceptable liquid fluorocarbon, and a visualizable label associated with the

fluorocarbon. The composition may further comprise an aqueous phase, and an emulsifier, wherein the composition is an emulsion and the liquid fluorocarbon comprises a fluorocarbon phase of the emulsion. In one embodiment, the label is a fluorescent label. Preferably, the label is hydrophobic and is associated with the fluorocarbon phase or with the emulsifier by lipophilic or hydrophobic interaction. The label may also be a visible dye.

The invention also includes a method for visualizing cells or tissue of an animal, comprising the steps of providing a fluorocarbon composition comprising a liquid fluorocarbon and a visualizable label associated therewith, administering the composition to the animal *in vivo*, permitting the composition to localize in cells or tissue, and visualizing the cells or tissue in which the fluorocarbon has localized. Preferably, the fluorocarbon composition is an emulsion and further comprises a continuous aqueous phase and an emulsifier, and the liquid fluorocarbon preferably comprises a discontinuous phase of the emulsion. In one embodiment, the discontinuous phase localizes in cells of the reticuloendothelial system. As above, the label may be fluorescent or may be a visible chromophore. The label is preferably hydrophobic and is associated with the discontinuous phase or the emulsifier by lipophilic or hydrophobic interaction. One particularly interesting use of this method is for visualizing lymph nodes or lymphatic vessels.

DETAILED DESCRIPTION OF THE INVENTION

The present invention utilizes fluorocarbons associated with a visualizable label, such as a visible or fluorescent dye or other chromophore, for marking tissues and cells. Unlike prior uses of fluorocarbons for radiographic, ultrasonic, or magnetic resonance imaging, the labelled fluorocarbons of the present invention may be directly visualized, with visible or ultraviolet light.

In one embodiment of the present invention, the fluorocarbon is in the form of a emulsion. Fluorocarbon

emulsions are well known. Such emulsions comprise an aqueous phase, an emulsifier, and a fluorocarbon phase. Both oil-in-water and water-in-oil emulsions can be prepared. These emulsion may be prepared using known techniques. See, e.g., U.S. Patent Nos. 4,987,154 and 4,865,836.

Suitable fluorocarbons for use in the present invention include any biologically compatible fluorocarbon. There are a number of fluorocarbons that have been disclosed for medical use. These fluorocarbons include bis(F-alkyl) ethanes such as $C_4F_9CH=CH_4CF_9$ (sometimes designated "F-44E"), $i\text{-}C_3F_9CH=CHC_6F_{13}$ ("F-i36E"), and $C_6F_{13}CH=CHC_6F_{13}$ ("F-66E"), cyclic fluorocarbons, such as C10F18 ("F-decalin," "perfluorodecalin" or "FDC"), F-adamantane ("FA"), F-methyladamantane ("FMA"), F-1,3-dimethyladamantane ("FDMA"), F-di-or F-trimethylbicyclo[3,3,1]nonane ("nonane"); perfluorinated amines, such as F-tripropylamine ("FTPA") and F-tri-butylamine ("FTBA"), F-4-methyloctahydroquinolizine ("FMOQ"), F-n-methyl-decahydroisoquinoline ("FMIQ"), F-n-methyldecahydroquinoline ("FHQ"), F-n-cyclohexylpurrolidine ("FCHP") and F-2-butyltetrahydrofuran ("FC-75" or "RM101").

Other fluorocarbons include brominated perfluorocarbons, such as 1-bromo-heptadecafluoro-octane ($C_8F_{17}Br$, sometimes designated perfluorooctylbromide or "PFOB"), 1-bromopenta-decafluoroheptane ($C_7F_{15}Br$), and 1-bromotridecafluorohexane ($C_6F_{13}Br$, sometimes known as perfluorohexylbromide or "PFHB"). Other brominated fluorocarbons are disclosed in U.S. Patent No. 3,975,512 to Long. Also contemplated are fluorocarbons having nonfluorine substituents, such as perfluorooctyl chloride, perfluorooctyl hydride, and similar compounds having different numbers of carbon atoms.

Additional fluorocarbons contemplated in accordance with this invention include perfluoroalkylated ethers or polyethers, such as $(CF_3)_2CFO(CF_2CF_2)_2OCF(CF_3)_2$, $(CF_3)_2CFO\text{-}(CF_2CF_2)_3OCF(CF_3)$, $(CF_3)CFO(CF_2CF_2)F$, $(CF_3)_2CFO(CF_2CF_2)_2F$, $(C_6F_{13})_2O$. Further, fluorocarbon-hydrocarbon compounds, such as, for example compounds having the general formula $C_nF_{2n+1}\text{-}C_{n'}F_{2n'+1}$, $C_nF_{2n+1}OC_{n'}F_{2n'+1}$, OR $C_nF_{2n+1}CF=CHC_{n'}F_{2n'+1}$, where n and n' are

the same or different and are from about 1 to about 10 (so long as the compound is a liquid at room temperature). Such compounds, for example, including $C_8F_{17}C_2H_5$ and $C_6F_{13}CH=CHC_6H_{13}$. It will be appreciated that esters, thioethers, and other variously modified mixed fluorocarbon-hydrocarbon compounds are also encompassed within the broad definition of "fluorocarbon" materials suitable for use in the present invention. Mixtures of fluorocarbons are also contemplated. Additional "fluorocarbons" not listed here, but having those properties described in this disclosure are additionally contemplated.

The emulsifier used in preparing the emulsion may be any suitable material, such as pluronic, nonionic surfactant, any of the fluorinated surfactants, or phospholipid emulsifiers, such as lecithin. Egg yolk phospholipid is particularly preferred. The surfactant typically comprises from about 2% to about 8% of the emulsion, w/v, and the fluorocarbon comprises from about 5% or 10% to about 90%, 100%, or 125%, w/v. (Because their density is about 2, the weight percentage of fluorocarbon in the emulsion can exceed 100%.)

The visualizable label may be selected from a wide variety of known labels. The label used in the present invention may be selected from the large number of conventional dyes, pigments, chromophores, and the like. It is preferred that the dye is lipophilic, or that it at least contains a lipophilic moiety. Alternatively, the dye may contain a fluorophilic moiety, and in certain instances, may be a fluorocarbon.

The labels of the present invention, for example, may include the following known chromophores: nitroso groups, nitro groups, azo groups, disazo groups, trisazo groups, polyazo groups, azoic groups, such as nitrosamine and diazo amino groups, stilbene groups, diphenylmethane (ketone imine) groups, triarylmethane groups, naphthyl groups, xanthene groups, thiazole groups, azines, oxizines, thiazines, amino ketones, indigoid groups, thioindigoid groups, and the like. Fluorinated derivatives of the foregoing are also

contemplated. Phosphorescent labels may be used. Many of the xanthene and naphthalene-type dyes are fluorescent. Fluorescein is a well known example.

One preferred category of fluorescent dye is disclosed in U.S. Patent No. 4,783,401. These materials are long chain lipid-like cyanine compounds, which are commercially available from Zynaxis Cell Science, Inc., Melvern, PA, under the trademark PKH-26™.

Although water soluble dyes may be used, such dyes remain largely in the aqueous phase and do not remain with the emulsion particles, except in the case of a water-in-fluorocarbon emulsion. On the other hand, the lipid-like labels such as those disclosed in U.S. Patent No. 4,783,401 remain with the fluorocarbon droplet, apparently as a result of lipophilic or hydrophobic interaction with the surfactant or with the fluorocarbon.

Another group of labels which may be advantageously used in the present invention are fluorescent, fluorinated aromatic molecules. Such molecules include octafluoronaphthalene, which has a fluorescent emission maximum at about 354 nm at an excitation wave length of 339 nm. Other fluorinated aromatics which would be soluble in the fluorocarbon phase and which are expected to be highly fluorescent may also be used, such as F-pyrene and F-anthracene.

Additional suitable labels may be selected, for example, from those listed in the Sigma-Aldrich Handbook of Stains, Dyes and Indicators, F.J. Green (Aldrich Chemical Company, Milwaukee, WI 1990).

The fluorocarbons of the present invention may be used in cell culture applications, or more importantly, *in vivo*. Neat fluorocarbon containing the label may be introduced into the lungs or the gastrointestinal tract of an animal. Gastrointestinal administration may aid in visualization during surgical treatment of obstructions, for example. Fluorocarbon emulsions of the present invention may be administered intravenously, intraperitoneally, subcutaneously, or directly into a lymphatic vessel. In each instance, the

labeled perfluorocarbon in the tissue in question may be utilized to label or visualize that tissue. Fluorocarbon emulsions tend to collect as a ring of enhancement around liver tumors, for example, 24 to 48 hours after IV administration. Fluorescent visualization of the fluorocarbon could facilitate surgical resection of such tumors. Moreover, it is contemplated that both fluorescent and visible dyes could be added to the same emulsion.

One particularly attractive method for using the technology of the present invention is in labeling the cells an organs of the RES system. Emulsified fluorocarbon materials tend to accumulate in RES organs such as the spleen and the lymph system. Moreover, fluorocarbon emulsions may be administered directly into lymphatic vessels (as in conventional lymphography). Alternatively, the lymphatic system, including vessels and lymph nodes, may be visualized by injecting fluorocarbon emulsion into tissues drained by the lymph nodes and vessels to be visualized. This technique is known as indirect lymphography. See, e.g., Wolf, et al, U.S. Patent No. 5,114,703. It is believed that phagocytic cells such as lymphocytes pick up and internalize the fluorocarbon particles from the emulsion. These particles are transported through the lymphatic system where they accumulate in lymph nodes and in the spleen.

Imaging of lymph nodes, for example, is not only valuable in research applications; it is also of significant value in surgery. Often, biopsy or lymphectomy procedures are performed in which it is important to identify and remove the tissue in question. Removal of lymph nodes is desirable, for example, in surgical treatments of certain tumors. The ability to visualize the lymph nodes during such surgery (either directly or through fluorescence) is an important advantage of the present invention. It is of similar value in post surgical examination of tissue removed from the patient.

The invention may be more fully understood with reference to the following example:

Example 1

Transport of Perfluorocarbon Emulsion From Subcutaneous Tissue to Regional Lymphatics

A 60% w/v perflubron emulsion (IMAGENT® LN, Alliance Pharmaceutical Corp., San Diego, CA) was combined with a fluorescent cyanine dye having long chain lipid-like characteristics (PKH-26, Zynaxis Cell Science, Inc., Melvern, PA). In particular, a 10^{-3} M stock solution of PKH-26 was added to the perflubron emulsion to provide a final concentration of PKH-26 of 10^{-5} M. The mixture was gently shaken in the dark for five minutes at room temperature (22°C). Subsequently, the mixture was added to the same amount of fresh rabbit plasma and was gently mixed for one minute to stop the staining reaction. This suspension had a final concentration of 30% perflubron, w/v. The resulting material was injected subcutaneously into the dorsal skin of the foot of anesthetized rabbits. Following injection, the foot was moved passively in a rotary direction at 0.3 Hz. Samples were collected from cannulated lower leg prenodal lymphatics over a period of two hours and were assayed for lymph flow rate, leukocyte count, and extracellular and intracellular labeled perfluorocarbon. Samples were taken at two hours, twenty four hours, and one week after injection. Following initial lymph sample collection, the foot was gently massaged for fifteen minutes and then the lymph measurements were repeated.

A fluorescent microscope was used to examine samples. The intracellular flux of the perfluorocarbon was measured as a function of the fluorescence of the sample. The measured results were:

2 hours	$3.7\pm3.7\times10^{-6}$ µg/hr
24 hours	$72\pm20\times10^{-6}$ µg/hr
24 hours	$61\pm16\times10^{-6}$ µg/hr

Extracellular flux was significantly greater at the initial stage:

2 hours	1.5 ± 0.4 µg/hr
24 hours	0.25 ± 0.07 µg/hr
1 week	undetectable

Examination of afferent rabbit lymph fluid using fluorescence microscopy revealed both internalized PKH-26-stained perflubron particles and freely suspended perflubron particles. These features were not visible under brightfield examination of the same field.

WHAT IS CLAIMED IS:

1. A fluorocarbon composition, comprising:

a physiologically-acceptable liquid fluorocarbon; and

a visible or fluorescent label associated with said fluorocarbon.

2. The composition of Claim 1, further comprising:

an aqueous phase; and

an emulsifier, wherein said composition is an emulsion and said liquid fluorocarbon comprises a fluorocarbon phase of said emulsion.

3. The composition of Claim 2, wherein said label is a fluorescent label.

4. The composition of Claim 2, wherein said label is hydrophobic and is associated with said fluorocarbon phase or with said emulsifier by lipophilic or hydrophobic interaction.

5. The composition of Claim 2, wherein said label is a visible dye.

6. A method for visualizing cells or tissue of an animal, comprising the steps of:

administering to said animal *in vivo*, a highly fluorinated liquid fluorocarbon having a visible or fluorescent label associated therewith, and permitting said composition to localize in cells or tissue; and

illuminating said cells or tissue with visible or ultraviolet light so as to visualize said cells or tissue in which said fluorocarbon has localized.

7. The method of Claim 6, wherein said fluorocarbon composition is an emulsion and further comprises a continuous aqueous phase and an emulsifier, and wherein said liquid fluorocarbon comprises a discontinuous phase of said emulsion.

8. The method of Claim 7, wherein said discontinuous phase localizes in tissue of the reticuloendothelial system.

9. The method of Claim 8, wherein said label is a fluorescent label.

10. The method of Claim 8, wherein said label is a visible dye.

11. The method of Claim 8, wherein said label is hydrophobic and is associated with said discontinuous phase or said emulsifier by lipophilic or hydrophobic interaction.

12. The method of Claim 8, wherein lymph nodes or lymphatic vessels are visualized in said visualizing step.

13. A physiologically-acceptable liquid fluorocarbon composition having a visible or fluorescent label associated therewith for use in visualizing cells or tissue.

14. The composition of Claim 13, in the form of an aqueous emulsion.

15. The composition of Claim 14, in which said emulsion comprises a continuous aqueous phase, a discontinuous fluorocarbon phase, and a surfactant, wherein said label is associated with said surfactant through hydrophobic or lipophilic interaction.

16. The composition of Claim 13, 14, or 15, wherein said use comprises administering said composition *in vivo* in a mammal and permitting said fluorocarbon and the associated label to localize in tissue of the reticuloendothelial system.

17. The composition of any one of Claims 13-16, for use in visualizing lymph nodes or lymphatic vessels.

18. Use of the composition of any one of Claims 1-5 or 13-17 in the preparation of a medicament for visualizing tissue of the reticuloendothelial system.

INTERNATIONAL SEARCH REPORT

Inte. ional Application No
PCT/US 94/02789

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US CHOWDHARY RK ET AL 'Influence of fluorocarbon emulsions on porphyrin-sensitised oxidation of histidine.' see abstract & PHOTOCHEM PHOTOBIOL, GB, APRIL 1990, VOL. 51, NO. 4, PAGE(S) 395-9	1-18
X	EP,A,0 272 933 (FUJI PHOTO FILM CO., LTD.) 29 June 1988 see claims; examples & JP,A,63 191 687 (...)	1-18

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
30 June 1994	07.07.94

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FED PROC, VOL. 34, NO. 6, PAGE(S) 1493-8, May 1975 Rosenblum WI 'Fluorocarbon emulsions and cerebral microcirculation.' see page 1494	1-18
X	PROG. COLLOID POLYM. SCI., VOL. 76, NO. TRENDS COLLOID INTERFACE SCI., 2, PAGE(S) 132-9, 1988 Haegel, F. H. et al 'Selective incorporation of dyes with fluorocarbon and hydrocarbon chains into coexisting micellar phases of sodium perfluorooctanoate and dimethyl tetradecyl aminoxide' see page 138	1-18
X	J AM COLL CARDIOL, VOL. 18, NO. 1, PAGE(S) 215-23., July 1991 Kolodgie FD et al 'Limitation of no reflow injury by blood-free reperfusion with oxygenated perfluorochemical (Fluosol-DA 20%).' see page 221 - page 223	1-18
X	ARCH OPHTHALMOL, VOL. 110, NO. 10, PAGE(S) 1468-71, October 1992 Azzolini C et al 'Interactions between light and vitreous fluid substitutes.' see figure 3	1-18
Y	GASTROENTEROL ENDOSC,, VOL. 28, NO. 4, PAGE(S) 769-777, 1986. MIZUIRI K et al 'LAPAROSCOPIC EXAMINATION OF PRIMARY BILIARY CIRRHOSIS AFTER ADMINISTRATION OF INDOCYANINE GREEN' see abstract; figures	1-18
Y	CELL BIOL INT REP,, VOL. 11, NO. 1, PAGE(S) 55-62, 1987. REED J A et al 'A FLUORESCENT DYE WHICH RECOGNIZES MATURE PERIPHERAL ERYTHROCYTES OF MYELOPROLIFERATIVE DISORDERS' see page 59 - page 61; figure 1	1-18
Y	J CELL BIOL,, VOL. 106, NO. 3, PAGE(S) 697-704, 1988. DEL BUONO B J et al 'RELATION BETWEEN THE ORGANIZATION OF SPECTRIN AND OF MEMBRANE LIPIDS IN LYMPHOCYTES' see page 698	1-18

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J TOXICOL ENVIRON HEALTH, VOL. 20, NO. 3, PAGE(S) 303-16, 1987 Levitt D et al 'Perfluorinated fatty acids alter merocyanine 540 dye binding to plasma membranes.' see page 307 - page 310	1-18
A	PFLUEGERS ARCH EUR J PHYSIOL,, VOL. 387, NO. 2, PAGE(S) 175-182, 1980. LUTZ J et al 'EFFECTS OF POTENTIAL BLOOD SUBSTITUTES PER FLUOROCHEMICALS ON RAT LIVER AND SPLEEN' see page 178 - page 180	1-18
A	REV FR TRANSFUS IMMUNO-HEMATOL,, VOL. 29, NO. 6, PAGE(S) 443-454, 1986 LUTZ J et al 'EVOLUTION INSIDE THE BODY AND FRACTIONAL EFFECT OF THE FLUOROCARBONS USED AS OXYGEN TRANSPORTERS'	1-18

INTERNATIONAL SEARCH REPORT

Intern...onal application No.
PCT/US 94/02789

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-18
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

In view of the large number of compounds, which are defined by the general definitions of the components, as mentioned in the claims, the search had t o be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and to the general idea

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

OBSCURITIES ETC.....

....underlying the apllication (see guidelines, Part. B, Chapt. III, paragraph 3.6).

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/US 94/02789

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0272933	29-06-88	JP-A-	63193343	10-08-88
		JP-A-	63193344	10-08-88
		JP-A-	63191687	09-08-88
		JP-A-	63159090	01-07-88
		US-A-	4832992	23-05-89
JP-A-63191687	09-08-88	EP-A,B	0272933	29-06-88
		US-A-	4832992	23-05-89